

GENETIC STUDIES ON *DROSOPHILA SIMULANS*. I. INTRODUCTION. HYBRIDS WITH *DROSOPHILA MELANOGASTER*¹

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[Received July 6, 1920]

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INTRODUCTION

Many attempts have been made to hybridize different species of *Drosophila*, but hitherto all but the combination to be described here have been unsuccessful. The present case is itself only to be considered a partial success, since the hybrids have so far all been completely sterile. Furthermore, since the two parent species are extremely similar and probably have identical chromosome groups, the data that are to be obtained from the study do not throw as much light as might be wished on many of the problems concerning the nature of the specific differences found in the genus *Drosophila*. Nevertheless, the investigation has led to interesting results bearing on such subjects as interspecific sterility, parallel mutations, chromosome maps, and sex determination.

DESCRIPTION OF *Drosophila simulans*

Drosophila simulans STURTEVANT (1919) is very similar to the well-known *D. melanogaster* Meigen (*ampelophila* Loew), and has been confused with it until recently. There is only one satisfactory character by which

¹ Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

the two species may always be separated; viz., the external male genitalia (figures 1 and 2). In living or relaxed pinned material the posterior process (P) of the genital tergite appears like a clam-shell in *D. simulans*, and like a small hook in *D. melanogaster*. The eggs (figures 3 and 4) are also characteristically different, but are more variable than the male genitalia, besides being less convenient to obtain. In addition to these characters, the cheeks are a little narrower and the eyes a little larger in

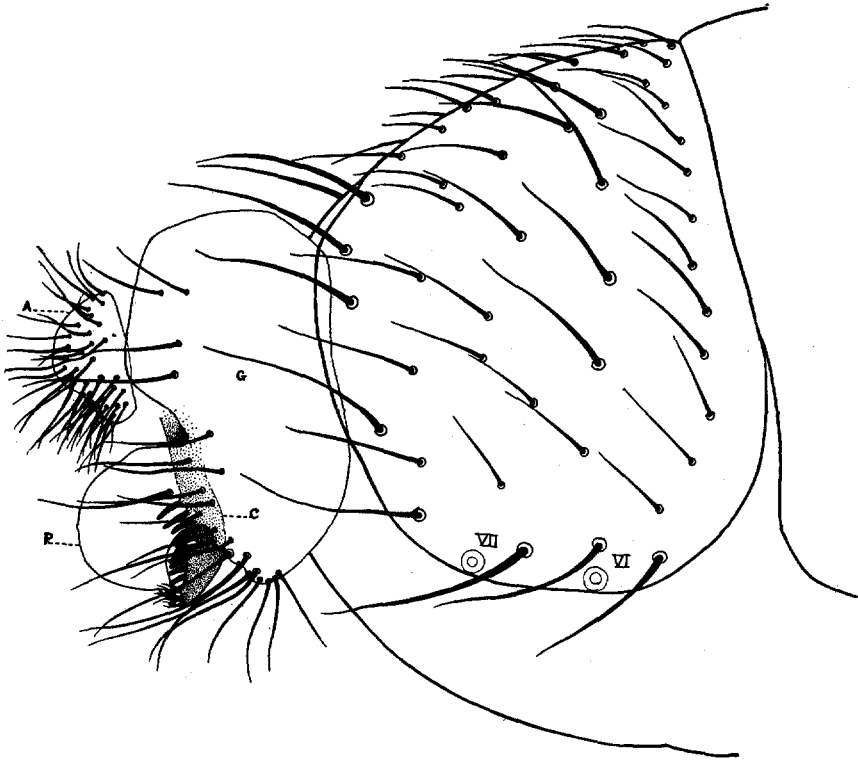


FIGURE 1.—External male genitalia of *Drosophila simulans*. From a specimen cleared and mounted in balsam. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of genital tergite; VI, VII, abdominal spiracles on fifth abdominal tergite.

simulans, and in general the newly hatched specimens have a more definite dark streak down the dorsal surface of the thorax (mesonotum) than has *melanogaster*. With a little experience one can usually separate most of the individuals in a mixed stock by the use of these characters. In addition there are other still more indefinite differences, that are observable when large series are compared. *Drosophila simulans* averages somewhat stouter in abdominal shape and darker in color than *melano-*

gaster. In mixed laboratory cultures *simulans* is apt to be smaller, though in pure cultures or in wild material there is no appreciable size difference.

Though experience will give one the impression that the two forms are distinct and should be classed as "good species," they are so close

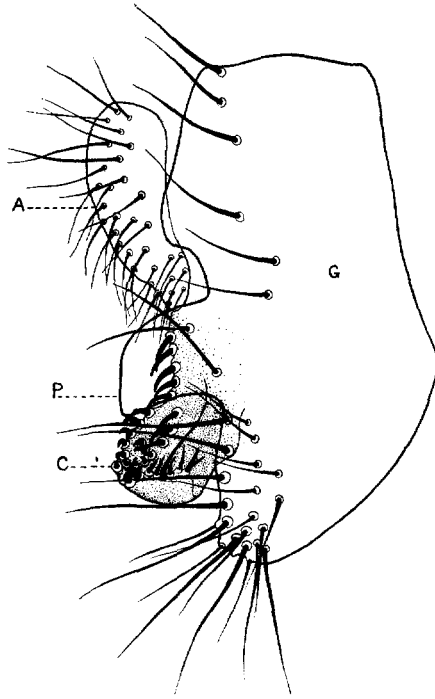


FIGURE 2.—External male genitalia of *Drosophila melanogaster*. Abbreviations as in Figure 1.

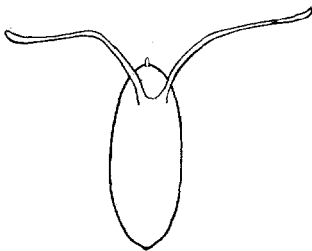


FIGURE 3.—Egg of *Drosophila simulans*.

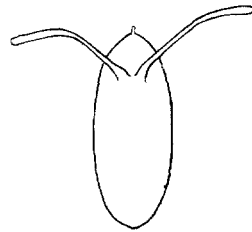


FIGURE 4.—Egg of *Drosophila melanogaster*.

that a technical description of *D. melanogaster* or a drawing of the style familiar to geneticists would pass equally well for *D. simulans*. So far as I have been able to discover, the mating and breeding habits and the length of the life-cycle are identical. Dr. C. W. METZ reports, after a preliminary study, that the chromosome groups are probably also identi-

cal. I am unable to distinguish the larvae, the pupae, or the female genitalia. The structure of the male genitalia and the sterility of the hybrids remain as the only valid grounds for making a specific distinction; but I think there can be no question that these grounds are sufficient.

DISTRIBUTION OF *Drosophila simulans*

I described *D. simulans* from stock collected at Lakeland, Florida. I have also seen specimens from New Hampshire, New York, Minnesota, Virginia, Georgia, Alabama, Costa Rica, Panama, and the Brazilian states São Paulo and Matto Grosso. Rather extensive collections from the Pacific coast and from the West Indies have so far not been found to contain any *simulans*, and it has not appeared in the Old World material that I have seen. *Drosophila melanogaster*, on the other hand, is evidently a cosmopolitan species. I have seen undoubted specimens, determined by examination of male genitalia, from American localities scattered from Nova Scotia to Oregon, California, Florida, Porto Rico, Costa Rica, Chile, and Brazil; and from Holland, Spain, New Zealand, Australia, and Hawaii. It is also recorded from Africa, India, and elsewhere.

Evidence has been presented by JOHNSON (1913) that indicates that *D. melanogaster* was introduced into this country from the tropics, and became common soon after 1870. The arguments advanced by JOHNSON are equally applicable to *simulans*, so far as they indicate the rarity or absence of the species before 1870, since the two forms have practically identical habits, and are so similar in appearance that even when JOHNSON wrote they had never been distinguished. The period at which *simulans* was introduced remains doubtful. It seems certain that QUACKENBUSH (1910) had specimens of it, derived from a stock collected at Woods Hole, Massachusetts, in 1908, and this is the earliest record known to me. The earliest specimen that I have actually seen was collected in Florida in 1912. It is quite probable, however, that an examination of large series of earlier material would show the species to have been established much earlier. The present distribution, outlined above, indicates that the species must have come from South or Central America.

DISCOVERY OF *Drosophila simulans*

At present *Drosophila simulans* and *D. melanogaster* are about equally common around New York City, in Florida, in Alabama, and in southwestern Brazil. Since the two are distinguishable only with some practice, and since a fresh wild stock usually contains both, it seems very surprising

that *simulans* was not accidentally discovered long ago in the course of genetic experiments with *melanogaster*. It must have been in the laboratory many times, but seems to have led to unexpected results only two or three times. The curious "unisexual broods" recorded by QUACKENBUSH (1910) were surely hybrid broods. Professor E. A. ANDREWS reported in correspondence some years ago that he had had difficulty in crossing a mutant race of *melanogaster* to a wild race collected at Baltimore, Md., and it now seems probable that the wild race was *simulans*. Finally, Mr. A. M. BROWN, in the COLUMBIA laboratory, obtained anomalous results with a wild race collected by me in Alabama, which ultimately led to the recognition of *simulans*. Mr. BROWN found that males of this race (now known definitely to have been *simulans*), when crossed to mutant races of *melanogaster*, gave only sterile female offspring.

Because of these results observed by Mr. BROWN, I asked Dr. C. W. METZ to send me a number of wild races from Lakeland, Florida, in the spring of 1919. These were tested by crossing to *melanogaster*, and about half of them were found to give unisexual broods. It was noticed that stocks that gave this result looked somewhat different from ordinary *melanogaster*, and had different eggs. Later, Dr. C. B. BRIDGES discovered the striking difference in the male genitalia. Not until many experiments had been made with the Florida race was it noticed that *simulans* also occurs in many other places. I have since shown by crosses, both to the Florida race and to *melanogaster*, that several New York races and one each from New Hampshire and from Minnesota, behave in the same way as does the Florida race. There can now be no question that the male genitalia, by which these later races were first identified, are a certain index as to the genetic behavior.

I have kept mixed stocks of the two species for several months, using the ordinary laboratory technique for keeping stocks of *melanogaster*, and have found that both species may persist together for at least five months. But in such mixed stocks *melanogaster* is usually far more numerous, and the few individuals of *simulans* are almost always small and pale. When the culture becomes old and dry *melanogaster* seems to be unfavorably affected more quickly, so that the percentage of *simulans* rises. Greater resistance to such unfavorable conditions may perhaps account for the fact that *simulans* maintains itself in nature in spite of the fact that, under favorable conditions, it is outdone by *melanogaster*. In the small numbers and small size of the *simulans* specimens from mixed stocks we apparently have the reasons why it was not discovered sooner, for when one selects flies for mating he usually picks out large vigorous

ones, and such a procedure would almost always lead to the selection of *melanogaster*. Furthermore, even if *simulans* was occasionally selected it would usually fail to cross, and the absence of offspring would simply be ascribed to sterility.

Drosophila melanogaster ♀ × *D. simulans* ♂

This cross was first made by QUACKENBUSH (1910), to judge from his account. It was also made by Mr. A. M. BROWN, as stated above. I first obtained it in May, 1919, and have since repeated it many times. If the mother is an ordinary XX individual the result is that *only female offspring are produced*. Hundreds of offspring have been obtained from matings of this type, without the production of males.² These hybrid females are completely sterile (with *simulans*, *melanogaster*, *obscura*, or hybrid males) and have rudimentary gonads, as QUACKENBUSH first showed.

These females always have the sex-linked characters expected of females from the cross. If the mother carries recessive sex-linked genes that are not present in the father, the corresponding recessive characters are never present in the hybrid daughters. This is true for all the recessive mutant characters tested, 12 in number. If sex-linked recessives are present in the *simulans* father, these also do not appear in the hybrid females. Three such have been tested. It follows that the hybrid females have two X chromosomes, and are not transformed males.

It has been shown by BRIDGES (1916) that females of *melanogaster* that have a Y chromosome (XXY) produce exceptional offspring of two types, due to the production of XX and Y eggs, respectively. The first (XX) type of eggs, if fertilized by Y sperm, produce daughters that get all their sex-linked genes from their mother, and are therefore matroclinous exceptions to the usual behavior of sex-linked characters. The second (Y) type of eggs, if fertilized by X sperm, give rise to sons that get their sex-linked genes from their father, and are thus patroclinous exceptions to the usual behavior of sex-linked characters. In order to further analyze the sex-ratio of the hybrid families, I have crossed XXY *melanogaster* females by *simulans* males. The result has been that *only the regular daughters and the exceptional sons are produced*. The other two classes, regular sons and exceptional daughters, are entirely absent.

² The results to be presented below indicate than an occasional male, due to primary non-disjunction, is to be expected. One male obtained in the cross involving scarlet *simulans* (to be described in part III of these studies) may have been of this nature.

Two experiments of this type have given the same result, and have produced a total of 128 regular females and 59 exceptional males.³

Hybrid males produced in this way are sterile, and have rudimentary testes. They are in no way distinguishable from the hybrid males obtained from the reciprocal mating. They have the sex-linked characters of the father, and are identified as non-disjunctional exceptions by this fact.

Drosophila simulans ♀ × *D. melanogaster* ♂

The reciprocal hybrids are more difficult to obtain. They have so far been produced most often from yellow mothers, except in one mating made by QUACKENBUSH. This mating has been made most often by Prof. T. H. MORGAN (1920), but I have made it successfully a few times. Usually the *only offspring produced are regular sons*, but MORGAN records having sometimes obtained also a few regular daughters, especially as the cultures became old. I have on one occasion used *simulans* females from a stock known to carry extra Y's, and obtained a hybrid female that was undoubtedly a non-disjunctional exception.⁴

As in the case of the reciprocal mating, all these hybrids were completely sterile.

ANALYSIS OF RESULTS

The results of the four types of interspecific matings may be tabulated as follows:

<i>melanogaster</i> ♀ (XX)	× <i>simulans</i> ♂	= regular ♀ ♀.
<i>melanogaster</i> ♀ (XXY)	× <i>simulans</i> ♂	= regular ♀ ♀ + exceptional ♂ ♂.
<i>simulans</i> ♀ (XX)	× <i>melanogaster</i> ♂	= regular ♂ ♂ + a few regular ♀ ♀.
<i>simulans</i> ♀ (XXY)	× <i>melanogaster</i> ♂	= regular ♂ ♂ + exceptional ♀ ♀.

Regular females will probably occur in the last mating in small numbers, as in the third mating. It is possible that other classes may appear, but none of them, aside from the exceptional males of the last mating, can survive in nearly as large a percentage of cases as do the types noted.

Analysis of these results indicates that the egg cytoplasm must play a part in determining what types survive. We may represent this cytoplasm by the symbol C, and denote the origin of it and of the chromo-

³ The large number of exceptions is due to the fact that a strain of "high non-disjunction" was used, and does not indicate a higher viability of the exceptional males.

⁴ The nature of this female was certain because she had the characteristic appearance of a hybrid, and because she was known to have received a *melanogaster* third chromosome but not a *melanogaster* X chromosome.

somes by sub-letters (m for *melanogaster*, s for *simulans*). The types investigated may then be tabulated as follows:⁵

♀		♂
$C_m X_m X_s$,—lives		$C_m X_m Y_s$,—dies
$C_m X_m X_s Y_m$,—lives		$C_m X_m Y_m Y_s$,—dies
$C_m X_m X_m Y_s$,—dies		$C_m X_s Y_m$,—lives
$C_s X_m X_s$,—usually dies		$C_s X_s Y_m$,—lives
$C_s X_m X_s Y_s$,—usually dies		
$C_s X_s X_s Y_m$,—lives		

The Y would not be expected to affect the result, and may probably be disregarded. The above formulae then simplify as follows:

♀		♂
$C_m X_m X_s$,—lives		$C_m X_m$ —dies
$C_m X_m X_m$,—dies		$C_m X_s$,—lives
$C_s X_m X_s$,—usually dies		$C_s X_s$,—lives
$C_s X_s X_s$,—lives		

This table presents many anomalous points. The difference between the two kinds of regular females ($C_m X_m X_s$ and $C_s X_m X_s$) can only be due to a difference in the cytoplasm of the mother, since the chromosomes of these two types must be identical.⁶

That hybrid females should develop in *melanogaster* cytoplasm only in case they carry a *simulans* X is surprising. This result must be due in part to the autosomes (or to the *simulans* Y), since $C_m X_m X_m$, that dies as a hybrid, is the formula of the normal *melanogaster* female. In general, it appears that hybrids develop only if they carry a *simulans* X, but that in the presence of *simulans* cytoplasm a *melanogaster* X usually inhibits development even though a *simulans* X is also present.

FATE OF $C_m X_m$ MALES

It has been assumed in the preceding discussion that the missing classes of hybrids die. It has been shown, by a consideration of the distribution of the sex-linked characters, that they are not changed over into any of the other classes. The possibility of selective fertilization is the only remaining alternative to the assumption that the individuals belonging to the missing classes are formed but die before they reach the adult stage. The evidence now to be presented shows that in the hybrid

⁵ The autosomes are alike in all cases (one member of each pair from each parent) and have therefore been left out of the formulae.

⁶ It is, of course, probable that the nature of this egg cytoplasm is determined in the first instance by the chromosomes of the female in which the eggs develop.

cultures from *melanogaster* ♀ × *simulans* ♂, many individuals die in the larval stage; and there is every reason to believe that these represent the missing males.

An eosin vermilion forked *melanogaster* female was mated to a forked *simulans* male. Larvae were observed in about five days, and the pair was removed to a fresh vial. A small amount of food was supplied, and the eggs laid on it were counted and removed, individually, to fresh food. Forty-seven eggs collected in this way gave rise to 14 adults, all of them hybrid forked females as expected. An attempt was made to count the pupa-cases after the adults had all emerged, but this was not found possible. It was clear from the attempt, however, that most of the pupae gave rise to adults, since only a few unhatched puparia could be discovered. Evidently, then, there is heavy mortality in the egg or larval stage. Observation of the isolated eggs indicated that larvae hatched from most of them. A few days after hatching it was noticeable that the larvae were of two sizes;—one type seemed to have grown normally, while the other was much smaller. It had previously been observed that a few larvae were usually present in a hybrid culture at the time adults ceased emerging, and that a number of hybrid larvae died without pupating. These observations indicate that the male larvae grow slowly and never pupate. Their history has not yet been followed in detail, nor has the fate of the other missing classes of hybrids been determined.

SEX-RATIOS OF OTHER SPECIES HYBRIDS

Numerous examples of anomalous sex-ratios among species hybrids are on record. These are evidently due to different causes, as will appear from an examination of a few examples.

When the domestic cow is fertilized by the male bison, the male offspring have a hump, derived from the bison, which is so large that it usually prevents their being born alive. The female hybrids lack this hump, and are born normally (BOYD 1914, etc.). Here, then, an abnormal sex-ratio is brought about at the time of birth by causes external to the hybrid itself,—i.e., by the structure of the mother.

GUYER (1909) has collected data on species hybrids among certain birds, and has shown that there is a decided excess of males in F_1 . Although it is possible that some of the individuals were in reality females that had developed male plumage as a result of imperfect development of their ovaries, the evidence indicates a real excess of males. RIDDLE (1916) has recorded the reverse relation (excess of females) for the cross

Streptopelia risoria × *S. alba* (species of doves) under certain conditions. The cross involves a sex-linked gene, and should give only dark males and white females. Examination of RIDDLE's data for the sex and color of the hybrids shows that, contrary to RIDDLE's own conclusion, the excess of females is not due to "changing over" of males. The female hybrids failed to receive a maternal Z chromosome and would therefore be expected to be females. The data also show that there is not a high enough mortality of embryos to account for the deficiency of males. The only remaining alternative explanation is that, under the conditions of the experiment, the Z chromosome went to a polar body at reduction oftener than it remained in the egg.

GOLDSCHMIDT (1916, 1917, 1919), HARRISON (1919), and others working with Lepidopterous hybrids have often obtained broods that were largely or entirely of one sex. HARRISON has shown that in at least one instance there is not a high enough mortality to account for the results; and both authors have presented evidence indicating strongly that the results are due to a "changing over" of the sex of half the individuals. Crosses in the same groups often produce intersexes, that are more or less intermediate between males and females. These intersexes commonly replace one of the expected sexes, while the other is present in the expected proportions. Intersexuality may be very slight or more and more marked. The unisexual broods evidently represent the final stage where one sex has been completely changed over into the other. The nature of this change is not yet satisfactorily explained; but it is clear that the anomalous sex-ratios are due to causes different from those operating in RIDDLE's doves or in the cow-bison hybrids. The intersexes will be discussed again, in connection with the *simulans* intersexes (part III of these studies).

The *melanogaster-simulans* hybrid sex-ratio evidently represents still a fourth type. It is not due to a changing over of sex, as shown by the distribution of the sex-linked genes. It is not due to a preferential maturation of the egg, since the female *Drosophila* is not heterozygous for sex. It is not due to external mechanical difficulties, since both types of larvae emerge from the eggs. The cause is an internal one, but acts merely by killing certain classes of larvae.

DESCRIPTION OF HYBRIDS

In general, *melanogaster-simulans* hybrids, of all types that have been observed, are intermediate between the parent species in those characters in which the parents differ. The male genitalia are shown in figure 5.

The width of the cheeks is also intermediate, as is the streak in the dorsal surface of the thorax. Since the hybrid females do not produce eggs, their egg-filaments cannot be examined.

In several respects the hybrids differ from both parents. The complete sterility of both sexes has already been referred to. It is connected with the rudimentary condition of the gonads. The ovaries especially are very minute, and in the testes no sperm have been seen. The hybrids

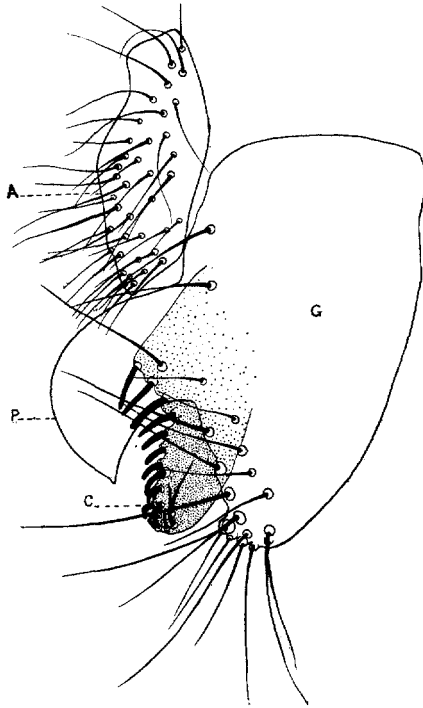


FIGURE 5.—External male genitalia of hybrid male. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of the genital tergite. The upper part of the first genital tergite was broken off in this specimen; this part of the plate is normally shaped as in the two parent species.

(more especially the females) frequently have fewer dorso-central and scutellar bristles than the number (4 of each sort) that is normal for each parent species. All of these bristles are sometimes missing, though in both pure species individuals with extra bristles are more frequent than those with bristles missing, and I have never seen wild specimens in which more than two were missing. Other bristles on the thorax (noto-pleurals, supra-alars, post-alars) are also often missing in the hybrids but not in the pure species. Another peculiarity that is not infrequent among the

hybrids (though less common than the missing bristles) is a broken or missing cross-vein. In the same category belongs abnormally banded abdomen. Both of these characters are very rare in wild individuals of each parent species. None of these characteristics of the hybrids suggests any related species of fly known to me.

These characters that appear in the hybrids but not in either parent species must be due to complementary genes carried by the two species. Since they are evidently dominant genes, it may be surmised that F_2 , were it possible to obtain it, would include individuals with numerous other new characters, due to recessive complementary genes.

SEXUAL SELECTION

A small series of observations indicates strongly that females of *melanogaster* or of *simulans* are far more likely to mate with males of their own species than with those of the other species. This is probably the reason why so many cross-matings fail to produce offspring. Both kinds of males will court females of either species, apparently indiscriminately; and that it is the females that are responsible for the small percentage of successful cross-matings is also indicated by the fact that normal *simulans* females have seldom been successfully crossed to *melanogaster* males. QUACKENBUSH used a female that had been subjected to heat, and nearly all later crosses have been from yellow females. I have shown (STURTEVANT 1915) that yellow females of *melanogaster* are more easily mated with by *melanogaster* males than are wild-type females.

Sexual selection, then, is one means whereby the two species are kept from crossing. It would, however, probably be ineffective if fertile hybrids were produced when cross-mating does occur.

Hybrids, of both sexes, have been seen to mate with both parent species. No sufficient data are at hand to show what preferences, if any, are shown by the hybrids, or how readily the parent species will mate with them.

SUMMARY

1. *Drosophila simulans* is a species closely similar to *D. melanogaster* in structure, habits, and chromosome group.
2. The two species may be crossed, though many cross-matings fail, apparently because females do not readily allow males of the other species to mate with them.
3. *Drosophila melanogaster* ♀ × *D. simulans* ♂ produces only female offspring, unless the mother is XXY in constitution. In the latter case

non-disjunction occurs, and regular daughters and exceptional sons are produced.

4. *Drosophila simulans* ♀ × *D. melanogaster* ♂ usually produces only male offspring, but females sometimes come in small numbers. One exceptional female, due to non-disjunction, has also appeared.

5. The regular females from these two matings are of the same chromosome constitution, but in one case they live and in the other they usually fail to emerge. This relation can only be accounted for by supposing that the egg cytoplasm influences the result.

6. Hybrids survive only if they carry a *simulans* X. In the presence of *simulans* egg cytoplasm and a *melanogaster* X, survival is not usual even if a *simulans* X is also present.

7. In the cross of *melanogaster* ♀ × *simulans* ♂, evidence is presented indicating that the regular males die in the larval stage.

8. The sex of the hybrids corresponds in all cases to their chromosome constitution. XX is female, X is male.

9. Hybrids of all classes are completely sterile, and have rudimentary gonads.

10. The hybrids are intermediate between the parent species in all respects in which the latter differ.

11. The hybrids often show some characters (absence of certain bristles, missing or broken cross-vein, abnormally banded abdomen) not present in either pure species.

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